Modes of Action of Trichloroethylene for Kidney Tumorigenesis

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This article focuses on the various models for kidney toxicity due to trichloroethylene (TCE) and its glutathione-dependent metabolites, in particular *S*-(1,2-dichlorovinyl)-L-cysteine. Areas of controversy regarding the relative importance of metabolic pathways, species differences in toxic responses, rates of generation of reactive metabolites, and dose-dependent phenomena are highlighted. The first section briefly reviews information on the incidence and risk factors of kidney cancer in the general U.S. population. Epidemiological data on incidence of kidney cancer in male workers exposed occupationally to TCE are also summarized. This is contrasted with cancer bioassay data from laboratory animals, that highlights sex and species differences and, consequently, the difficulties in making risk assessments for humans based on animal data. The major section of the article considers proposed modes of action for TCE or its metabolites in kidney, including peroxisome proliferation, α_{2u}-globulin nephropathy, genotoxicity, and acute and chronic toxicity mechanisms. The latter comprise oxidative stress, alterations in calcium ion homeostasis, mitochondrial dysfunction, protein alkylation, cellular repair processes, and alterations in gene expression and cell proliferation. Finally, the status of risk assessment for TCE based on the kidneys as a target organ and remaining questions and research needs are discussed. *Key words*: α_{2u}-globulin, cell proliferation, cysteine conjugate β-lyase, genotoxicity, kidney, oxidative stress, peroxisome proliferation, renal cancer, trichloroethylene. — *Environ Health Perspect* 108(suppl 2):225–240 (2000).
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This article focuses on the various models for kidney toxicity due to trichloroethylene (TCE). In particular, focus will be on the role and quantitative importance of the glutathione (GSH)-dependent (i.e., cysteine conjugate β-lyase [β-lyase]) pathway in generation of reactive metabolites that have been associated with various cytotoxic and carcinogenic responses to either TCE or to the GSH conjugate *S*-(1,2-dichlorovinyl)glutathione (DCVG). A major focus will be factors and responses contributing to or leading to renal tumorigenesis, as this topic has been of major interest in human health risk assessment. Areas of controversy will be highlighted, including discussions of species differences in toxic responses and rates of generation of reactive metabolites and dose-dependent phenomena. Key metabolites that have been associated with cytotoxic and carcinogenic effects in laboratory animals will be identified, and their relevance to humans will be evaluated.

The first section briefly reviews information on the incidence of kidney cancer in the general U.S. population. Incidence and risk factors for kidney cancer in general are briefly discussed, and then data on incidence of kidney cancer after exposure to TCE are evaluated. Limited epidemiological data are available for renal cancer in humans exposed to TCE. Studies of TCE-induced renal cancer in laboratory animals highlight sex and species differences and, consequently, the difficulties in making risk assessments for humans based on the animal data. The second section will briefly review the toxic and reactive metabolites of TCE that may be important in TCE-induced renal toxicity. The subject of TCE metabolism is dealt with in greater detail in the article on "Metabolism of Trichloroethylene"(*1*). The third and major section of this paper considers proposed modes of action for TCE in the kidneys. Several mechanisms are proposed, including peroxisome proliferation, α_{2u}-globulin nephropathy, genotoxicity, and acute and chronic cytotoxicity. The latter includes oxidative stress, alterations in calcium ion homeostasis, mitochondrial dysfunction, protein alkylation, cellular repair processes, and alterations in gene expression and cell proliferation. Finally, the status of risk assessment for TCE based on the kidneys as a target organ and remaining questions and research needs will be discussed.

Epidemiology of Kidney Cancer

Incidence and Mortality Rates of Kidney Cancer in the U.S. Population

This section summarizes data on the incidence of kidney cancer and mortality in the general U.S. population and the health consequences involved. Further, risk factors that may enhance susceptibility to kidney cancer are summarized. The discussion on the general properties of renal neoplasia is summarized from Brenner and Rector's *The Kidney, Fifth Edition* (*2*). Malignant neoplasms involving the renal parenchyma and renal pelvis may be primary or secondary in origin. Primary neoplasms are those that derive from transformation of renal cells, whereas secondary neoplasms are those that derive from metastases of tumors in other tissues. In the kidneys, the frequency of metastatic neoplasms is higher than that of primary tumors. Invasive disease accounts for 99% of all tumors. Renal-cell carcinoma accounts for approximately 85% of all primary renal neoplasms. Primary neoplasm of the renal pelvis or ureter accounts for 7–8% of renal neoplasms; nephroblastoma (Wilms tumor) accounts for 5–6% of the total; various sarcomas of renal origin account for the remainder of the primary tumors. Incidence rather than mortality is a better indicator of kidney cancer, since survival (5-years) is 58% for males and females combined for invasive disease; no differences in survival between sexes have been observed (*3,4*). Survival is even higher, 87%, for localized disease (*4*). Generally, the primary treatment for kidney cancer is surgical removal of the diseased kidney. It is rare that both kidneys are affected, and individuals can function quite well with one kidney. Renal-cell carcinoma is characterized by diverse and often obscure symptoms and may easily be mistaken for other diseases.

Controversy still exists about the relationship between renal-cell carcinoma and renal adenoma, as the two are not readily distinguishable on the basis of gross histologic, immunologic, or ultrastructural features. Both types of cells arise from the proximal convoluted tubules. However, renal adenomas are typically incidental findings at autopsy, usually as small, well-circumscribed lesions of the renal cortex, and renal adenocarcinomas are usually larger lesions.

Estimates for new cases of primary kidney cancer and other urinary cancer cases for

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males and females combined for 1996 and 1997 are 36,000 and 28,800, respectively, with 12,000 and 11,300 deaths, respectively (*5,6*). Incidence and mortality rates for cancer as the primary kidney disease (kidney and renal pelvis combined, since data are not available for a specific subsite) for the most recent time period are for 1995. In 1995, the age-adjusted incidence rate was 9.2 per 100,000, with rates in males twice those of females (*4*). Incidence has been increasing over time, with the increase in nonwhites much greater than in whites (*4*). Mortality rates reflect the same pattern as incidence; however, rates are lower due to relatively good survival. The age-adjusted mortality rate in 1995 was 3.6 per 100,000, and showed a gradual increase over the past 20 years (*4*). The increasing mortality among nonwhites over time was greater than that among whites (*4*). Recent analysis of the National Cancer Database (*3*) suggests that kidney cancers are being diagnosed at an earlier stage of the disease and with greater precision.

Risk Factors

A number of risk factors have been identified within recent years for renal-cell carcinoma in one national and one international multicenter case–control study. The focus of this discussion is on renal-cell carcinoma, since it is the most predominant form of kidney cancer. Risk factors are primarily hypertension status, diet, family history, and personal lifestyle factors. Occupational factors have been subject only to limited study; most studies do not uniquely identify specific chemical exposures. Renal-cell cancer is not usually considered an occupationally related cancer, as are lung and bladder cancer (*7*).

In the latter cases, exposures are assessed according to job category or class of agent. The epidemiologic evidence examining TCE exposure, specifically, will be discussed in the next section.

The strongest evidence for which a causal association can be inferred is between renal-cell carcinoma and smoking. Statistically significant elevated risks for renal-cell carcinoma have been consistently reported in several studies (*8–11*) with cigarette smoking. Risks increased with increasing duration and number of cigarettes (pack-years), adding further support for a causal association. As with other smoking-related cancers, risk is reduced among long-term former smokers. One other study (*12*) additionally reports an association between cigarette smoking and carcinoma of the renal pelvis and ureter. McLaughlin et al. (*7*) estimate that between 24 and 30% of all renal cancer deaths are attributable to smoking.

Associations between renal-cell carcinoma and other etiologic agents are more variable and less definitive than those for cigarette smoking. Limited data suggest that long-term use of phenacetin-containing analgesics is associated with an increased renal-cell carcinoma risk (*13–16*). Abuse of analgesics containing phenacetin has been causally associated with renal pelvis and ureter cancer (*17*). Having a relative with a previous diagnosis of renal-cell carcinoma has been identified in two studies (*7,10*). Not unexpectedly, a past history of kidney stones or kidney disease is also associated with renal-cell carcinoma, although these observations may be biased due to differential recall of past kidney disease between cases and controls (*7*). Limited evidence exists for an association between the use of diuretics and other antihypertensive agents and renal-cell carcinoma (*18–22*). Additionally, a history of hypertension has been identified as another risk factor (*21*). Hypertension, diuretic use, and taking antihypertensive medication are highly correlated, making it difficult to identify which factors may be more important. Three recent studies (*9,23,24*) provide support for the hypothesis that diets high in meat or fried meats are associated with an increase in risk for renal-cell cancer and that diets high in vegetable and fruit content are protective. Body mass index was found to be a risk factor among females, and to a lesser extent, among males, with rate of weight change appearing as an independent risk factor (*25*). Last, fairly consistent findings have been reported for a modest, positive association between the number of births and risk for renal-cell carcinoma and a protective effect with use of oral contraceptives (*26–28*).

Only now are the human studies taking a more serious examination of possible associations between renal-cell carcinoma and occupational exposures. Most information on occupation comes from case–control studies where exposure is only crudely characterized. Associations between kidney cancer and occupational exposures or job categories that have been reported in at least one study are the following: truck drivers and those exposed to gasoline (*22,29,30*); employment in the iron or steel industry (*31*); with exposure to gasoline (*29–32*), aviation fuel (*33*), or other petroleum products (*31*); insecticides or herbicides (*22*); asbestos (*31*); cadmium (*31*); and dry-cleaning solvents (*31*). The large case–control studies are adequate for raising hypotheses regarding possible associations; however, they are severely limited when they are used alone to support statements regarding possible causal associations.

Cohort studies of petroleum workers have not reported elevated risks with kidney cancer (*34*). Alternatively, case–control studies of kidney cancer are not as consistent; two case–control studies report associations with gasoline exposure (*31,32*), whereas a recent case–control study of kidney cancer nested among male petroleum workers did not show any associations with exposure to petroleum hydrocarbons (*35*). Some data indicate that selected subgroups of males, such as downstream and distribution workers and service station workers, may have elevated kidney cancer risk (*36–38*). The epidemiologic observations are of interest since male, but not female, rats exposed to unleaded gasoline developed renal-cell carcinoma. Further experimentation showed that male rats were unique in developing an accumulation of α_{2u} after exposure to many halogenated hydrocarbon solvents and this was necessary and essential for development of kidney cancer (see below). This mechanism is considered to be only relevant in male rats and not in humans; thus, renal-cell carcinoma developing by this mechanism is not considered a hazard to humans (see discussion in section on α_{2u}-globulin nephropathy below).

An additional risk factor for renal-cell cancer is genetic, and involves the von Hippel-Lindau (VHL) tumor suppressor gene (*39*). So-called VHL disease is a hereditary cancer syndrome that is characterized by the development of vascular tumors of the central nervous system (CNS) and retina, pheochromocytomas, pancreatic islet cell tumors, endolymphatic sac tumors, clear-cell renal carcinomas, and benign cysts affecting a variety of organs (*40*). VHL disease can be caused by germline mutations of the VHL gene located on chromosome 3p25. Renal involvement is central to VHL disease and has emerged as the most prevalent cause of death (*41*). However, VHL mutations in kidney cancer can be somatic as well as germline (*42*).

Occupational and Other Human Exposures to TCE and Renal Disease, Including Cancer

There have been few studies that have examined exposure to TCE and development of kidney disease. One case report exists of acute renal failure, with normal liver function, in a male worker opening bins containing 7.5 L of a nearly pure solution of TCE (*43*). The studies of Nagaya et al. (*44*) and Rasmussen et al. (*45*) suggest kidney dysfunction among male workers with exposure to TCE (*44*) or solvents (*45*). Insight into possible TCE-associated injury is limited, since only 30% of the participants studied by Rasmussen et al. (*45*) had TCE exposure. Additionally, both studies were of a prevalence or cross-section design and the most recent exposure was only crudely assessed. No information was given regarding historical exposures, a time period considered more relevant for assessing kidney toxicity. Another study of a small group of male metal degreasers in Sweden (*46*) observed no increase in *N*-acetyl-β-glucosaminidase (NAG) excretion into urine, and

concluded that TCE was not nephrotoxic at low exposure levels.

Brüning et al. (*47*) obtained blood and urine samples from a 17-year-old male who ingested approximately 70 mL TCE in a suicide attempt. The patient showed the well-known symptoms of acute solvent intoxication, including CNS depression, tremor, general motor restlessness, and finally coma. Cardiotoxicity, as evidenced by sinus tachycardia and ectopias, was also observed, which is consistent with the known ability of halogenated hydrocarbons to sensitize the myocardium to adrenergic transmitters. The patient did not exhibit any of the standard clinical parameters of nephrotoxicity, such as increases in glucose and total protein excretion or increases in serum creatinine or blood urea nitrogen (BUN), during the initial 24 hr after hospitalization. However, significant increases in beta-2-microglobulin (β-2-MG), NAG, and albumin excretion were observed, indicating tubular damage. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis of urinary proteins showed the increased presence of low molecular weight proteins in the 10,000- to 50,000-Da range, providing additional, more specific evidence of tubular damage. This study is, therefore, the first to demonstrate that a single, oral dose of TCE can produce nephrotoxicity in humans.

Brüning et al. (*48*) performed a retrospective study on 39 workers who were exposed to high levels of TCE from 1956 to 1975, to investigate possible persistent nephrotoxic effects of TCE. Concentrations of GSH *S*-transferase (GST)-α, urinary excretion of which is a marker of proximal tubular damage, were elevated in the urine of TCE-exposed workers but not in that of control workers. Urinary excretion of GST-π, which is a marker of distal tubular damage, was not elevated in TCE-exposed workers. The authors concluded that chronic exposure to high doses of TCE causes persistent changes to the proximal tubules and that GST-α can be used as a marker for quantitation of the extent of renal damage.

The International Agency for Research on Cancer (IARC) (*49*) recently evaluated the epidemiologic evidence on TCE; kidney cancer incidence and mortality were examined among other types of cancer. The overall evaluation of cancer risk reported in the IARC monograph (*49*) from dry cleaning in general does not suggest an increase in the risk for cancer of the kidney, although the results of proportionate mortality studies and of a case–control study indicate an increase in risk associated with a history of work as a dry cleaner. Elevations in relative risks for mortality from cancers of the urinary bladder, esophagus, pancreas, lung, liver, and gall bladder, and non-Hodgkin's lymphoma were cited. Overall, the review group stated that there is *limited evidence* in humans for the carcinogenicity of occupational exposures in dry cleaning, concluding that dry cleaning entails exposures that are *possibly carcinogenic to humans* (Group 2B). For TCE specifically, the overall evaluation was that there is *limited evidence* in humans for carcinogenicity of trichloroethylene and *sufficient evidence* in experimental animals for the carcinogenicity of TCE, providing for an overall conclusion that TCE is *probably carcinogenic to humans* (Group 2A).

Eight studies reviewed by IARC examined the relationship between TCE (or TCE with other halogenated solvents) exposure and kidney cancer mortality or incidence. Most studies were of occupational exposures. Two studies examined mortality and groundwater exposure to TCE. Case–control studies of kidney cancer were also evaluated. IARC placed greater weight on observations from three cohort studies (*50–52*) where no elevations in kidney cancer risks were noted, and the two case–control studies reported divergent results. IARC (*49*) noted that a study of male German cardboard manufacturing workers exposed to TCE (*53*) observed five cases of renal cancer compared to none in the comparison population. Four cases were of renal-cell carcinoma and the fifth of cancer of the renal pelvis. The Henschler et al. report (*53*) presented, for the population studied, standardized incidence rates (SIR) for kidney cancer, using rates from the Danish Cancer Registry and from the former German Democratic Republic as comparisons. For each comparison, a statistically significant elevation in the SIR for kidney cancer was noted. IARC (*49*) considered these findings to be a cluster and concluded that kidney cancer among these male workers needed further study. The report of Henschler et al. (*53*) has spurred much controversy (*54–56*); even if the workers studied represent a cluster, this warrants further study since several human carcinogens were first recognized through cluster investigations.

Two other reviews (*57,58*) were conducted after that of IARC. Both examined a slightly different set of studies than those of IARC and from each other. Whereas Weiss (*58*) did not explicitly draw conclusions about the kidney, McLaughlin and Blot (*57*) concluded that there is neither consistent nor convincing evidence to support a causal relationship between TCE exposure and renal-cell cancer.

Three reports have recently appeared in the literature (*59–61*) that, when taken together, add further evidence about an association between TCE and kidney cancer. Blair et al. (*59*) updated the study of Spirtas et al. (*52*) on the Hill Air Force Base (Ogden, UT) maintenance workers exposed to TCE (*62*) and Morgan et al. (*60*) examined mortality in another group of aircraft maintenance workers. Whereas both Blair and Morgan concluded that small numbers of kidney cancer deaths limited their findings, both studies reported elevated risks (not statistically significant) among those with TCE exposure. Additionally, risk appeared to increase with increasing cumulative exposure in the study of Morgan et al. (*60*) but not in that of Blair et al. (*59*). Further, the case–control study of Vamvakas et al. (*61*) noted a statistically significant odds ratio with occupational exposure to TCE when compared with accident controls with no TCE or PER exposure. Risk increased with increasing exposure (from an odds ratio of 6.6–11.4).

The reports of Brüning et al. (*47,48, 63–66*) strengthen the epidemiologic evidence between TCE and kidney cancer. Brüning et al. (*65*) reported a larger risk for kidney cancer among those individuals with specific GST isozymes M1 and T1, raising the question of whether differential metabolism of TCE may produce a greater quantity of or more toxic metabolites. In the second study (*64*), renal-cell carcinoma tissue from 23 patients [many of whom were cases in the study of Vamvakas et al. (*61*)] with occupational history of very high exposure to TCE were analyzed for somatic mutations in the VHL gene. By contrast, renal-cell carcinoma tissue from patients who were not exposed to TCE exhibited a significantly lower (33–55%) VHL mutation frequency. The VHL mutations in these individuals are somatic and not germline, since none of these patients had a family history of VHL disease (*67*). Further, mutational analysis of the VHL gene showed a transition at nucleotide 454 (C > T) at codon 81 in 35% of the patients, which Brüning et al. (*66*) believed to be different from findings in patients with germline or sporadic (no TCE exposure) renal cell carcinomas. Together, these data indicate that the VHL gene is a susceptible and specific target for TCE-induced renal carcinogenesis and that these results provide further evidence that the kidneys are targets in humans who are occupationally exposed to high doses of TCE. One note of caution, however, is that industrial-grade TCE has often contained other contaminating chemicals. It is unclear what impact these contaminants may have had on the development of renal cell carcinoma.

Bioassays for Kidney Cancer in Laboratory Animals

TCE is known to cause cancer in laboratory animals. Evidence of TCE carcinogenicity in experimental animals includes findings of low incidences of renal tumors in several strains of male rats when the compound is administered by oral gavage or by inhalation.

Statistically significant increases in renal tumors have not been observed in female rats, although occasional occurrences of a rare renal tubular-cell tumor have been reported in some studies that also reveal increases in these tumors in males. Kidney tumors are not associated with exposure to TCE in mice or hamsters, and not all carcinogenicity studies in rats are consistent in demonstrating renal tumors (*68–73*).

In a National Toxicology Program (NTP) bioassay, groups of 50 male and 50 female rats were administered 0, 500, or 1,000 mg/kg/day high-purity TCE by gavage in corn oil vehicle 5 days a week for up to 103 weeks. Fifty male and 50 female rats were used as untreated control groups. Three high-dose males developed renal tubular-cell adenocarcinoma. Two animals in the low-dose male group developed renal tubular-cell adenoma. Renal tubular-cell tumors are rare in this strain of rats. Other observed kidney tumors in this study included a transitional-cell carcinoma of the renal pelvis in a low-dose male, a carcinoma of the renal pelvis in a high-dose male, a transitional-cell papilloma of the renal pelvis in an untreated control male, and a rare tubule adenocarcinoma in a high-dose female. Toxic nephrosis was observed in 96 of 98 treated males and in all treated females, but not in vehicle-control rats. Survival of treated male rats was significantly reduced: 35 vehicle controls, 20 low-dose rats, and 16 high-dose males were alive at terminal sacrifice. Because of reduced survival, the statistical methods that adjust for animals at risk or intercurrent mortality provide more meaningful results for risk analysis than unadjusted statistical methods. Increase in kidney tubular-cell adenocarcinoma incidence in high-dose males was (3/16 or 19%) compared to vehicle-control males (0/33 or 0%) at the end of the study. The small increase of renal tubular-cell adenocarcinomas observed in high-dose male rats at terminal sacrifice was statistically significant by life table analysis and incidental tumor tests when pairwise comparisons were made between the dosed group and the vehicle-control group. The life table analysis regards tumors in animals dying prior to terminal kill as being the cause of death. The incidental tumor test regards these lesions as nonfatal. Unadjusted statistical methods such as the Fisher Exact Test and Cochran-Armitage Trend Test compare the overall incidence rates directly.

The NTP conducted a similar chronic oral carcinogenicity bioassay on ACI, August, Marshall, and Osborne-Mendel rat strains. Groups of 50 males and 50 females were administered 0, 500, or 1,000 mg/kg/day purified TCE in corn oil by gavage, 5 days/week, for 103 weeks. Fifty male and 50 female rats were used as untreated controls. The survival of all treated groups was significantly lower than that of controls. Oral administration of TCE in this study was associated with an increased incidence of renal tubular-cell adenomas and adenocarcinomas. Because of reduced survival, toxicity, and deficiencies in conduct of the study, however, the NTP concluded that the study was inadequate for assessing either the absence or presence of carcinogenicity, although they reviewed the results as indicating renal toxicity in all tested rat strains, including the Fischer 344 (F344) rat studied in their 1986 bioassay. There was no difference in kidney toxicity between males and females of any strain. The histopathologic evaluations of the kidneys of all five strains of rats have been summarized (*72–75*). The toxic nephropathy observed in these studies clearly does not present as the spontaneous lesion occurring in aging rats. Rather, the lesions are characterized by cytomegaly, karyomegaly, and toxic nephrosis of the tubular epithelial cells in the inner renal cortex. These lesions were not observed in control animals. Severity of cytomegaly was found to be proportional to duration of dosing as observed in animals that died early. The 1990 NTP report describes a statistically significant increase in renal adenomas in male Osborne-Mendel rats and the increase in renal adenocarcinomas in F344 rats previously reported in the 1988 bioassay report. Five of the six renal adenomas in low-dose male Osborne-Mendel rats occurred among the 17 rats alive at the end of the study. One rare renal tubular-cell adenocarcinoma was seen in a high-dose male Osborne-Mendel rat in this study. The NTP also noted the finding of one rare tubular-cell adenocarcinoma in a male Osborne-Mendel rat in a previous study (*70*).

Henschler et al. (*68*) administered pure TCE (stabilized by an amine base) by inhalation at 0, 100, and 500 ppm for 6 hr/day, 5 days per week, for 18 months to NMRI mice, F344 rats, and Syrian hamsters of both sexes. No significant increase in tumor formation was observed in any species or dosing groups, except for malignant lymphomas in female mice. The authors concluded that their findings provide no indication for a carcinogenic potential of pure TCE.

Maltoni et al. (*69*) exposed groups of 130–145 Sprague-Dawley rats to 0, 100, 300, or 600 ppm TCE (99.9% pure, containing no epoxide) 7 hr/day, 5 days per week, for 104 weeks. Animals were observed for their lifetimes. There was no difference in survival or mean body weight among the rats exposed to airborne concentrations of TCE when compared to control rats. Renal tubular-cell adenocarcinomas were observed in four high-dose animals, three males and one female. No tumors of this type were reported in the lower-dose groups, in the concurrent control animals, or in the more than 50,000 historical control Sprague-Dawley rats used in that laboratory. Maltoni et al. (*69*) also administered 0, 50, or 250 mg/kg TCE in olive oil by gavage to groups of 30 male and 30 female Sprague-Dawley rats, 4–5 days per week for 52 weeks. The animals were observed for their lifetimes, and none of them developed kidney tumors.

Although the kidney tumor increases in male rats are not all statistically significant, the findings are generally considered to be biologically significant and important in an assessment of potential human hazard because renal tumors in rats are rare. That is, such tumors are not often seen in large numbers of historical control animals. In some instances, however, chemically induced male rat kidney tumors are species- and gender-specific and, therefore, of little importance in human hazard evaluation. Thus, of particular interest is the objective of the analysis presented in this paper to focus attention on available data that address whether TCE-induced male rat kidney tumors may be the result of mechanisms or a mode-of-action operative, in theory, in humans.

Based on the above-mentioned long-term carcinogenicity studies, both Goeptar et al. (*76*) and Brüning et al. (*63*) have suggested that chronic cellular injury is a necessary prerequisite for production of renal tumors. In a study of hospital patients diagnosed with renal-cell cancer, those who had previous, documented exposure to TCE had evidence of average to severe tubular damage at higher rates than those who had no previous exposure to TCE (*63*).

The issue of nephrotoxicity and chronic cellular injury in relation to development of renal tumors deserves further comment. It is clear from the studies in rodents that renal-cell tumors do not occur in the absence of some kidney damage. The kidneys are highly susceptible to damage due to their high blood flow and the presence of myriad transport mechanisms that allow renal epithelial cells, particularly those of the proximal tubule, to concentrate bloodborne and filtered chemicals to high intracellular levels. Moreover, renal epithelial cells contain a large array of bioactivating enzymes, both those that are similar to the xenobiotic-metabolizing enzymes in the liver and those that are unique to the kidneys, that metabolize chemicals to reactive and toxic species (*77,78*). Although one can conclude that the kidneys are a minor target organ *in vivo* and that risk is low, because the incidence of renal tumors is typically low, the incidence of nephrotoxicity is much higher. The hypothesis of Goeptar et al. (*76*) and Brüning et al. (*63*) that renal cellular injury is a *prerequisite for producing renal tumors*

suggests further that the injury is followed by induction of repair processes, which then leads to cellular proliferation. However, if the extent of the nephrotoxicity is greater, renal cells will be irreversibly injured and the tissue will not be capable of activating repair processes. In that case, then, cellular proliferation and carcinogenesis will not be observed, and the risk based on tumor incidence will be underestimated.

In the sections that follow, potential biochemical mechanisms or modes of action of TCE in producing renal-cell cancer will be discussed.

Toxic and Reactive Metabolites of TCE That May Be Important in Renal Toxicity of TCE

Oxidative Pathway of TCE Metabolism

TCE is metabolized by two general metabolic pathways, an oxidative pathway whose initial step is catalyzed by cytochrome P450 (P450), and a GSH-dependent pathway whose initial step is catalyzed by GST. Key oxidative metabolites that may be associated with toxicity and tumorigenesis include trichloroacetate (TCA), dichloroacetate (DCA), and chloral hydrate (CH). Although formation of these metabolites by P450 and other oxidative enzymes has generally been emphasized in considerations of hepatic metabolism and liver injury, renal proximal tubular cells also possess many of the same isoforms of P450 that are found in the liver parenchymal cells, including CYP2E1, which is the primary isoform believed to catalyze oxidation of TCE (*79,80*). Hence, these metabolites can be formed by renal enzymes and may play some role in TCE-induced renal injury.

Little investigation into the role of P450-derived metabolites of TCE in TCE-induced renal injury has been undertaken. It is believed that TCA and DCA are the primary metabolites that cause hepatic injury and liver tumors. Although these two acids were not acutely cytotoxic in isolated rat kidney cells, they were potent inhibitors of mitochondrial state 3 respiration (*81*). This suggests that their formation in kidney could lead to alterations in renal cellular function and, if the kidneys were exposed chronically to even small amounts of TCA or DCA, to cytotoxicity or transformation and tumorigenesis. Further study of the potential role of TCA or DCA in renal effects resulting from TCE exposure are necessary.

GSH Conjugation Pathway of TCE Metabolism

It is generally believed that metabolites of TCE derived from the GSH conjugation pathway are responsible for the majority of the renal effects of TCE. Key metabolites that may be associated with toxicity and tumorigenesis include DCVG, *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), and DCVC sulfoxide. Special aspects of renal and cellular handling of GSH conjugates and their associated metabolites that make the kidneys particularly susceptible to acute and chronic injury will be briefly discussed. These aspects include tissue-specific transport mechanisms and enzymatic reactions. Although these points are discussed in greater detail in the article on "Metabolism of Trichloroethylene" (*1*), it is important to state them here because of their critical importance in understanding mechanisms of renal injury and carcinogenesis.

There is no question that DCVC and the reactive species that are generated from its metabolism by either the β-lyase or the cysteine conjugate *S*-oxidase, are nephrotoxic (*76*). The critical question then is not whether the GSH-derived metabolites of TCE can produce nephrotoxicity, because the answer to that is definitively yes, but rather whether the doses of TCE or DCVG that get to the kidneys are sufficient to generate enough reactive species to produce regulatory or toxic alterations. The ability of these reactive metabolites to produce nephrocarcinogenicity or renal-cell cancer, however, has not been directly demonstrated. Cancer bioassays with DCVG or DCVC are needed to address this question. Additionally, it has usually been assumed that the majority or all of TCE conjugation with GSH occurs in the liver, as tissue concentrations of the GSTs are much higher there than in other tissues, and a fraction of the DCVG formed or other metabolites, such as the mercapturate, eventually gets to the kidneys by interorgan translocation pathways. Lash et al. (*81,82*) recently showed, however, that the kidneys of rats can also catalyze conjugation of TCE with GSH to form DCVG, although at rates that are 10–20% of those in the liver. Hence, it is not necessary to invoke interorgan pathways to explain formation of nephrotoxic metabolites from TCE.

Recently, Bruckner and colleagues (*83*) developed a physiologically based pharmacokinetic (PBPK) model for estimation of tissue concentrations of TCE after intra-arterial injection, bolus oral gavage, and inhalation exposure. A summary of estimates of maximal tissue concentrations and area under the curve (AUC) values for TCE in liver and kidney by the three exposure routes is given in Table 1. There are several striking findings in these estimates. With either the intra-arterial injection or oral gavage exposure route, maximal tissue concentrations and AUC values in the kidneys, although lower than those in the liver, are still significant, being approximately 60% or 20%, respectively, of those in the liver. By inhalation, however, both C_{max} and AUC values for the kidneys were approximately 3-fold higher than those for the liver. Actual measurements of these values agreed very closely with the PBPK estimates. These data demonstrate that the kidneys are exposed to significant amounts of the parent chemical and, taken together with data reported by Lash et al. (*81,82*), that formation of oxidative or GSH-derived metabolites from TCE can occur within the kidneys at appreciable rates.

Formic Acid Excretion in TCE-Exposed Rats

Recently, Green et al. (*84*) have suggested an alternative mechanism for kidney damage induced by long-term exposure to TCE. Rats that were given either single or multiple doses of TCE, either by gavage or inhalation, excreted large amounts of formic acid in the urine. The amount of formic acid excreted after a single exposure to 500 ppm TCE was reported to be comparable to that observed after a 500 mg/kg dose of formic acid itself. Exposure of rats to 250 or 500 ppm TCE over 28 days resulted in urinary excretion of large amounts of formic acid, increased urinary excretion of ammonia, and decreased urinary pH, but no morphological damage to liver or kidney. Furthermore, formic acid was shown not to be a metabolite of TCE. Based on these results and the known nephrotoxicity of formic acid, the authors concluded that urinary excretion of high amounts of formic acid after exposure to TCE may contribute to the renal damage attributed to GSH-derived metabolites of TCE.

Although the concept that metabolic perturbations resulting in formation of high amounts of formic acid as a mode of action by which TCE produces kidney damage is an intriguing hypothesis, this proposal cannot account for much data that indicate a requirement for β-lyase–dependent metabolism of TCE to produce nephrotoxicity. Moreover, there is no evidence in the literature that formic acid can produce renal tumors. Hence, although formic acid formation may contribute to TCE-induced renal damage, this is not likely to be a significant mode of action in TCE-induced kidney tumorigenesis.

Table 1. TCE pharmacokinetic parameter estimates for liver and kidney after exposure of rats by intra-arterial injection, bolus oral gavage, or inhalation.[a]

	Liver		Kidney	
Exposure	C_{max}	AUC	C_{max}	AUC
Intra-arterial injection	5.3	88	3.2	56
Bolus oral gavage	9.1	124	1.9	33
Inhalation	0.4	46	1.5	159

[a]Male Sprague-Dawley rats were exposed to TCE by one of three routes: intra-arterial injection (7.2 mg/kg body weight), bolus oral gavage (8 mg/kg body weight), or inhalation (2-hr inhalation of 50 ppm TCE). Units of C_{max} = μg/g. Units of AUC = μg × min/mL. Data reported in Varkonyi et al. (*83*).

Proposed Modes of Renal Carcinogenicity of TCE in Rodents and Their Relevance to Humans

This section discusses four major mechanisms by which TCE may cause renal tumors. These include peroxisome proliferation, accumulation of the male rat-specific protein α_{2u}-globulin, direct genotoxicity, and acute or chronic toxicity. In some cases, information is available using both TCE and the presumed penultimate toxic metabolite DCVC as the treatment agent. In other cases, however, only data with DCVC as the treatment agent are available, so that inferences and extrapolations relating these data back to the parent compound TCE must be made. Under the subheading of acute and chronic mechanisms of nephrotoxicity and cytotoxicity, several biochemical processes are discussed with regard to their relevance in DCVC- and/or TCE-induced kidney tumorigenesis. Although much of the mechanistic data have been obtained with rodents or with tissue or cells derived from rodents, emphasis is made where data have been obtained in humans or from human cells or tissue.

Peroxisome Proliferation

Generation of chloroacetates from TCE may arise by both oxidative metabolism (i.e., cytochrome P450) and by further metabolism of DCVC. Since chloroacetates are known to produce hepatic peroxisome proliferation, renal enzymes also generate chloroacetates, and proximal tubular epithelial cells are relatively rich in peroxisomes, peroxisome proliferation is a plausible mode of action for TCE. As discussed above and in the article on "Mode of Action for Liver Tumorigenesis" (*85*), TCA and DCA have been identified as the metabolites of TCE that are responsible for peroxisome proliferation in male rat liver (*86,87*).

In relation to the mode of action of TCE in the kidney and the potential risk for humans, three central issues are *a*) whether significant formation of TCA and DCA occurs in the kidneys, *b*) whether peroxisome proliferation is induced to a significant extent in the renal proximal tubules, and *c*) whether this mechanism occurs in humans. Regarding the first issue, studies are underway to quantify oxidative metabolism of TCE in renal cells and microsomes from rat, mouse, and human kidney. However, no information is available at present to allow assessment of the quantitative significance of this pathway in the kidneys from rodents or humans.

The second issue, namely that of whether TCE induces peroxisome proliferation in the kidneys, has been addressed directly in only one study (*88*), whereas other studies have assessed peroxisome proliferation with either a related chemical or with a presumed metabolite of TCE (*89*). Goldsworthy and Popp (*88*) investigated the ability of TCE and perchloroethylene (PER) to induce peroxisome proliferation in liver and kidney of rats and mice, using increases in cyanide-insensitive palmitoyl-CoA oxidation activity as a marker enzyme. Both TCE and PER elevated enzyme activity in mouse liver and kidney, whereas only TCE elevated activity in rat liver and kidney. They concluded that there is an association between peroxisome proliferation and hepatic tumors in mice but suggested that peroxisome proliferation does not correlate with halogenated hydrocarbon-induced renal carcinogenicity. Odum et al. (*89*) studied the role of TCA generated from PER in tumorigenesis and peroxisome proliferation in liver and kidneys from male and female F344 rats and $B6C3F_1$ mice. Due to the pharmacokinetics of PER, male mice were exposed to 6.7-fold higher amounts of TCA than male rats, and peroxisome proliferation was only observed in male mouse liver. Hence, they concluded that peroxisome proliferation does not play a role in the apparent carcinogenicity of PER in male rat kidney.

Other studies on peroxisome proliferation suggest that peroxisomes are differentially regulated in liver and kidney (*90,91*). For example, di-(2-ethylhexyl)phthalate (*92*), clofibrate (*93*), ciprofibrate (*94*), ethionine (*95*), and valproate (*96*) all caused marked peroxisome proliferation in rat livers and caused smaller extents of peroxisome proliferation in rat kidneys. Diets high in fish oil were able to modestly induce peroxisome proliferation in mouse livers but not in mouse kidneys.

Hence, from these studies we can conclude that *a*) if renal levels of accumulation of TCA from TCE are similarly lower than hepatic levels of accumulation of TCA as was found for PER, then peroxisome proliferation is unlikely to be important in the kidney for TCE; and *b*) renal peroxisomes are generally less responsive to peroxisome proliferators than hepatic peroxisomes, which also makes a role for peroxisome proliferation in TCE-induced nephrocarcinogenicity unlikely.

As for the third issue, there has been much debate about the significance of peroxisome proliferation in human liver. A prevailing view is that peroxisome proliferation is likely to be largely a rodent-specific response, with primate species including man being markedly less responsive to drug-induced peroxisome proliferation than rodents, and that peroxisome proliferation either does not occur or occurs to much a smaller extent in humans (*97,98*).

α_{2u}-Globulin Nephropathy

α_{2u} is the major component of the urinary protein load in male rats and is unique to male rats, although homologous proteins exist in other species, including humans. Renal proximal tubules reabsorb protein from the glomerular filtrate, and toxicants or pathological conditions that interfere with this process cause an excessive accumulation of α_{2u} in lysosomes of renal proximal tubular cells. However, a similar phenomenon has not been observed in female rats or in other species. A number of chemicals, many of them halogenated organic solvents, have been shown to cause the so-called hyaline (protein) droplet nephropathy in male rats. The proposed steps for the induction of nephropathy and renal tumors by chemicals that induce α_{2u} nephropathy include the following:

- Protein droplets containing α_{2u} increase in number and size in renal proximal convoluted tubular cells of male rats exposed to certain halogenated hydrocarbons. α_{2u} is a low molecular weight protein (M_r = 18,700 Da) that is synthesized in the liver of mature male rats under androgenic control, and is not synthesized in the livers of immature male rats, the livers of female rats, or the livers of either sex of several other species, including mice or humans. Hydrocarbons or their metabolites that induce the response bind irreversibly to α_{2u}, resulting in the lysosomal degradation of the complex (*99*).
- The excessive accumulation of reabsorbed proteins in secondary lysosomes of the renal proximal convoluted tubules (S_2 segment) is then thought to cause lysosomal dysfunction and cellular necrosis.
- Intratubular granular casts of necrotic cellular debris then accumulate at the junction of the pars recta of the proximal tubules (S_3 segment) and the thin loop of Henle.
- Regenerative cellular proliferation is then induced in response to the loss of cells from the S_2 segment of the proximal tubules.
- The increased cellular proliferation is then thought to cause development of renal-cell tumors due to increases in DNA damage in replicating cells.

Goldsworthy et al. (*100*) examined the ability of TCE, PER, and pentachloroethane to induce α_{2u} accumulation, protein droplet nephropathy, and cellular proliferation in the kidneys of male and female F344 rats. Both PER and pentachloroethane produced accumulation of α_{2u} in male but not female rats, and this correlated with both protein droplet nephropathy and increases in cellular proliferation. In contrast, TCE did not induce increases in α_{2u} and did not stimulate cellular proliferation. Hence, they concluded that the mechanism of TCE-induced nephrocarcinogenicity must differ from that of PER.

Furthermore, Melnick (*101*) proposed that the accumulation of α_{2u} in kidneys of male rats due to exposure to certain halogenated hydrocarbons and the consequent nephropathy and induction of cellular proliferation do not explain the male rat-specific nephrocarcinogenicity of these chemicals. Rather, Melnick (*101*) suggested that by binding halogenated hydrocarbons, α_{2u} may act as a transport protein to increase delivery of the hydrocarbon to the target tissue. The paper by Melnick (*101*) generated some controversy, as it contrasted directly with the mechanism proposed by Lehman-McKeeman et al. (*99*) and others. In response to Melnick's proposed alternative mechanism, the proponents of the generally accepted mechanism published a commentary (*102*) arguing that no experimental evidence exists to support the alternative mechanism and that the widely accepted mechanism of α_{2u}-mediated renal injury is supported. They additionally emphasized that the decision of the U.S. Environmental Protection Agency (U.S. EPA) to accept the widely accepted mechanism allowed them to appropriately conclude that the α_{2u} phenomenon is largely male rat specific and is not relevant to humans. Melnick (*103*) replied that there is evidence for an alternative mechanism of action and recommended that rather than conclude that this process is irrelevant to humans, one should conclude that species differences in transport and disposition of chemicals alters delivery of toxic chemicals to the target organ. He emphasized that functionally analogous proteins to α_{2u} are present in humans.

Nonetheless, the prevailing view is that the α_{2u} hypothesis is male rat specific and that this mechanism is not relevant to humans (*104*). The α_{2u} found in male rats is structurally related to a group of transport proteins, many of which are found in humans. The proteins of this family of about 20 proteins, called lipocalins, are similar in molecular weight, have some sequence homology, and some are known to have tertiary structure similar to α_{2u}. The only protein with a known physiological function is retinol-binding protein, although all the proteins of the family are thought to be carriers of lipophilic molecules. Since concentrations of these homologous proteins in human urine are well below those of α_{2u} that are found in male rats, it is highly unlikely that enough protein could accumulate in human kidney to produce the same sort of hyaline droplet nephropathy that is seen in the male rat (*105*). Hence, accumulation of α_{2u} would not appear to be relevant for TCE-induced nephrocarcinogenicity and is likely not relevant for human health risk assessment for TCE.

In spite of the decision of the U.S. EPA and the report of the National Research Council, considerable controversy still exists in the scientific community regarding both the mechanism of renal carcinogenesis induced by chemicals that produce α_{2u} and the relevance of this to human health risk assessment. This point is illustrated by a the publication of a recent series of articles and commentaries (*106–112*).

Genotoxicity

This section presents evidence for the role of DNA damage and mutations in TCE-induced renal carcinogenesis. Data have been obtained with either TCE or with the presumed, penultimate nephrotoxic and nephrocarcinogenic metabolite, DCVC, on bacterial mutagenesis, DNA adduct formation (both nuclear and mitochondrial), unscheduled DNA synthesis (UDS), DNA strand breaks, cell proliferation, and oncogene activation. Data on UDS, cell proliferation, and oncogene activation are presented below in the section "Acute and Chronic Nephrotoxicity and Cytotoxicity."

Evidence for DNA damage or mutagenesis with TCE administration. Studies on the mutagenicity of TCE have been performed in bacteria, fungi, yeast, and in cultured mammalian cells. TCE was not mutagenic in a bacterial mutagenicity assay using *Salmonella typhymurium* TA100 (*113*) nor were *his*$^+$ revertants detected in *S. typhymurium* TA100 in the presence of rat kidney S9 fraction (*114*). TCE was weakly mutagenic in the mold *Aspergillus nidulans* when it was in the growing phase only (*115*). In cultured mammalian cells, TCE did not induce sister chromatid exchange in Chinese Hamster ovary cells (*116*) and did not induce DNA repair in primary cultures of rat hepatocytes (*117*). Furthermore, studies in lymphocytes of workers exposed to TCE did not provide any evidence of chromosomal damage at TCE exposure levels of up to 30 ppm (*118*). Hence, there does not appear to be any convincing evidence that TCE is mutagenic or genotoxic. However, it should be pointed out that there are many confounding factors in some of these mutagenicity studies, such as the presence of mutagenic stabilizers in the preparations of TCE, and that a thorough investigation of TCE-induced DNA damage in cells from the various target organs, particularly the liver and kidneys, has not been performed. An important consideration in evaluation of these results is the activity of enzymes of the mercapturate and β-lyase pathways in the bacterial strains and other systems used to detect mutations. A negative response may simply be due to the absence of the necessary enzymes for the complete metabolism of TCE.

Evidence for DNA damage or mutagenesis with DCVG or DCVC administration. Several studies have demonstrated that either DCVG or DCVC are mutagenic in bacterial strains by the Ames test. Dekant et al. (*119*) showed that DCVC, *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC), and *S*-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine (PCBC), the cysteine conjugates of TCE, PER, and hexachloro-1,3-butadiene (HCBD), respectively, are all mutagenic in three strains of *S. typhimurium* (TA100, TA2638, TA98) by the Ames test. Similarly, Vamvakas et al. (*120*) showed that both DCVG and DCVC are mutagenic in *S. typhimurium* TA2638. Moreover, both studies showed that mutagenicity was decreased by preincubation with aminooxyacetic acid (AOAA), demonstrating a requirement for metabolism by the β-lyase to generate a mutagenic molecule. Vamvakas et al. (*120*) also showed that the mutagenicity of DCVG was potentiated by addition of a rat kidney fraction that contains a high content of γ-glutamyltransferase (GGT), which is consistent with the presence of low GGT activity in the bacterial strain. Commandeur et al. (*121*) also compared the mutagenicity of the 1,2- and 2,2-isomers of DCVC and found that the 1,2-isomer was significantly more mutagenic than the 2,2-isomer and that this correlated with the 3- to 4-fold higher β-lyase activity with the 1,2-isomer. Hence, all three of these studies demonstrated the presence of at least some activity of the necessary enzymes, including the β-lyase, in the mutagenicity test systems.

More direct measures of genotoxicity of GSH-derived metabolites of TCE have also been obtained. Vamvakas and colleagues (*122*) observed UDS and micronucleus formation in Syrian hamster embryo fibroblasts, which were inhibited by AOAA, and UDS in LLC-PK_1 cells (*123*). In the study in LLC-PK_1 cells, an immortalized cell line derived from porcine proximal tubules, dose-dependent induction of UDS was also observed with TCVC and PCBC. In another study with LLC-PK_1 cells, Vamvakas et al. (*124*) showed that DCVC induced DNA double-strand breaks, which were attributed to activation of Ca^{2+}- and Mg^{2+}-dependent endonucleases, and an increase in poly(ADP)-ribosylation of nuclear proteins.

Green and Odum (*125*) compared the cytotoxicity and mutagenicity of a series of nephrotoxic cysteine conjugates to determine structural requirements for mutagenicity. Cysteine conjugates of HCBD, *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC), hexafluoropropene, TCE, and PER were compared. With this limited number of compounds, it became clear that conjugates of chloroalkenes were both nephrotoxic and mutagenic, whereas conjugates of fluoroalkenes were similarly nephrotoxic but were not mutagenic. Hence, a chlorine is required as a leaving group to generate the reactive

species that can bind to or otherwise alter cellular DNA.

Conclusions of a recent study by Völkel and Dekant (*126*) contrast with those above. These authors studied the reactivity of chlorothioketene (the ultimate reactive species generated from DCVC) with DNA bases in both organic and aqueous solvents. *S*-(1,2-Dichlorovinyl)thioacetate, which generates chlorothioketene, reacted quite poorly with cytosine in an aqueous solution. The authors concluded, based on theoretical considerations as well as the cytosine adduct results, that "experimental demonstration of DNA adduct formation in the kidney after administration of TCE or PER to rodents has to be considered very difficult, if not impossible." This conclusion implies that a genotoxic mechanism of action for DCVC is highly unlikely. However, there are at least two potential problems with the interpretation by Völkel and Dekant. First, an aqueous solution such as that used in their study does not adequately replicate the intracellular environment that would be found in the intact cell. Second, only adducts with cytosine were studied. It is possible that adducts with other bases might be energetically more favorable. Nonetheless, these results provide a starting point to question the viability of a genotoxic mode of action based on chemistry. Additional studies are necessary to more fully assess the chemical basis of DCVC-induced genotoxicity.

Acute and Chronic Nephrotoxicity and Cytotoxicity

A potential nongenotoxic mode of action for TCE involves repeated events of cellular necrosis and activation of repair processes that lead to cellular proliferation. This section reviews data on mechanisms of cytotoxicity induced by TCE or GSH conjugate-derived metabolites of TCE in renal proximal tubular cells from both rodents and humans. Both acute and chronic exposures have been performed, using both *in vivo* exposures and *in vitro* models such as freshly isolated cells and cell cultures. Most of the *in vivo* and *in vitro* studies aimed at elucidating biochemical modes of action in the kidney have focused on DCVG or DCVC rather than the parent compound, since most of the available data indicate that it is flux through the GSH conjugation pathway that generates the reactive species that are responsible for nephrotoxicity and potentially for nephrocarcinogenicity. In contrast, a limited number of *in vivo* or *in vitro* toxicity studies have been performed with TCE itself. Virtually all the published data on administration of TCE to laboratory animals have involved cancer bioassays. Consequently, only a few mechanistic studies using the parent chemical are available.

Acute and chronic nephrotoxicity:* in vivo *studies. Chakrabarty and Tuchweber (*127*) studied the acute nephrotoxicity of TCE in male F344 rats, administered either by an intraperitoneal injection of TCE in corn oil or by inhalation. By either route of administration, TCE produced elevations in urinary NAG, GGT, glucose excretion, and BUN, all of which are characteristic signs of proximal tubular damage. Increased excretion of high molecular weight protein in the urine was also detected, suggesting some glomerular injury.

Cojocel et al. (*128*) assessed the role of oxidative stress in TCE-induced nephrotoxicity after *in vivo* administration of TCE to male NMRI mice by intraperitoneal injection. TCE depleted renal cortical GSH content but not hepatic GSH content, and produced elevations in renal cortical content of malondialdehyde (MDA) and ethane expiration, which are indicators of lipid peroxidation. Under the same conditions, no changes were observed in hepatic MDA levels. A dose-dependent increase in BUN levels was also observed, confirming the decrement in renal function induced by TCE. Prior depletion of GSH content with buthionine sulfoximine enhanced the effect of TCE on renal cortical MDA content.

In a limited chronic toxicity study of DCVC in rats, Terracini and Parker (*129*) found large, abnormal nuclei in renal tubular cells. Jaffe et al. (*130*) performed a similar, more detailed chronic toxicity study in male Swiss-Webster mice. DCVC (0.01, 0.05, and 0.1 mg/mL) was administered in the drinking water over a period of up to 37 weeks. The two higher concentrations of DCVC produced a clear retardation of growth by 21 weeks and by 26 weeks, cytomegaly, nuclear hyperchromatism, and multiple nucleoli were found in cells of the pars recta of the proximal tubules. At later time points, renal tubular atrophy and interstitial fibrosis were observed. No effects were seen in the liver, consistent with the known target-organ specificity.

In vivo, acute exposures of laboratory animals to either DCVG or DCVC result in clear signs of renal proximal tubular injury: Elfarra et al. (*131*) found that both DCVG and DCVC administered to male F344 rats by intraperitoneal injections in isotonic saline resulted in elevations in BUN and urinary glucose excretion. Furthermore, inhibition of renal GGT activity with acivicin protected rats from DCVG-induced nephrotoxicity. In addition, both the β-lyase inhibitor AOAA and the renal organic anion transport inhibitor probenecid provided protection from DCVC, demonstrating a requirement for metabolism of DCVG to the cysteine conjugate by the action of renal GGT and dipeptidase, uptake into the renal cell by the organic anion transporter, and subsequent activation by the β-lyase. This conclusion was supported further by showing that the α-methyl analog of DCVC, which cannot undergo a β-elimination reaction due to the presence of the methyl group, was not nephrotoxic.

Darnerud et al. (*132*) showed similar findings in female C57BL mice, using covalent binding of radiolabeled DCVC to acid-insoluble renal tissue and histopathology as measures of nephrotoxicity. GSH depletion or addition of probenecid diminished DCVC covalent binding and nephrotoxicity, indicating a role for oxidative stress and organic anion transport in DCVC-induced nephrotoxicity (see below).

Several studies on the isomeric specificity of DCVC-induced nephrotoxicity showed that the L-isomer is more potent than the D-isomer (*133,134*) and that the 1,2-isomer is more potent than the 2,2-isomer (*135*). These results are consistent with the known enzymology of DCVC bioactivation and with the isomer specificity studies on DCVC-induced mutagenicity described above.

Acute cytotoxicity:* in vitro *studies. Chakrabarti and Tuchweber (*127*) showed that accumulation of aminohippurate by renal cortical slices, which is often used as an indicator of proximal tubular function, was inhibited at 24 hr after intraperitoneal administration of 22 mmol TCE/kg to male F344 rats.

In addition to the higher susceptibility of male rats to TCE-induced nephrocarcinogenicity and nephrotoxicity, isolated renal cortical cells from male F344 rats are more susceptible to acute cytotoxicity from TCE than cells from female rats. As shown in Figure 1, TCE caused a modest increase in lactate dehydrogenase (LDH) release from male rat kidney cells but had no significant effect on LDH release from female rat kidney cells. Similar, although more pronounced effects were observed with PER (Figure 1C, D). PER, which differs from TCE only by the presence of a fourth chlorine atom, undergoes bioactivation reactions similar to TCE, forming a GSH conjugate that through subsequent renal metabolism yields a nephrotoxic and nephrocarcinogenic reactive species (*136,137*). Hence, the results from the *in vitro* model system on sex dependence of susceptibility to TCE and PER agree with the *in vivo* data.

In contrast to these results with the parent compounds, kidney cells from male rats (*81,138*), or an established renal cell line (*139*) incubated with DCVG or DCVC exhibit much greater amounts of LDH release than the parent compounds. Moreover, as shown in Figure 2, DCVC-induced cytotoxicity is modestly higher in kidney cells from male rats compared to female rats, although

the dose–response relationship is rather complex. Hence, the same general sex dependence of susceptibility that was observed for the parent compound is observed for the penultimate, cytotoxic metabolite.

Preparations of renal tissue from the rabbit have also been used as *in vitro* models by some groups to study cysteine conjugate-induced nephrotoxicity. In general, the rabbit kidney cells or tubules seem to exhibit similar sensitivities to DCVC as those from the rat. Wolfgang et al. (*140*) found that DCVC was rapidly taken up by cortical slices from rabbit kidney and within 1 hr 40% of the DCVC was covalently bound to the tissue. Toxicity was evidenced by release of brush-border membrane enzymes (GGT, alkaline phosphatase) during the first 4 hr after exposure and by histopathology and electron microscopy. Within 4–8 hr, necrosis of the S_3 segments of the proximal tubules was evident, and this progressed by 12 hr to encompass all segments of the proximal tubule. Mitochondrial and brush-border membrane damage were evident biochemically within less than 1 hr and morphologically by 6 hr. Irreversible cellular injury was concluded to occur within 30 min. In two similar studies in suspensions of proximal tubular fragments from rabbit kidney, Hassall et al. (*141*) and Groves et al. (*142*) showed that DCVC produced the same types of effects as seen in kidney slices. Hassall et al. (*141*) also demonstrated an important role for tubular cell concentrations of GSH in modulating the ability of DCVC to inhibit tubular active transport, suggesting a role for oxidative stress as a mode of action for DCVC (see below). Groves et al. (*142*) also compared the cytotoxicity of DCVC with that of a nephrotoxic haloalkyl cysteine conjugate, TFEC, and found, similar to the mutagenicity studies described above, that haloalkenyl cysteine conjugates with chloride-leaving groups are more potent than haloalkyl cysteine conjugates with only fluoride-leaving groups.

Validation of the primary role of the β-lyase in the bioactivation and cytotoxicity of DCVG and DCVC in human kidney is supported by the purification of the enzyme activity from human kidney cytosol (*143*) and the characterization of DCVG- and DCVC-induced cytotoxicity in primary cultures of proximal tubular cells from human kidney (*144*). Activity of the purified enzyme and that in the primary cell cultures was sensitive to AOAA; cytotoxicity of DCVC was diminished by AOAA. Hence, the β-lyase pathway occurs in human proximal tubular cells and can mediate the cytotoxicity of DCVC. In contrast to these results, Cummings and Lash (*145*) recently found that acute cytotoxicity induced by DCVC in freshly isolated human proximal tubular cells

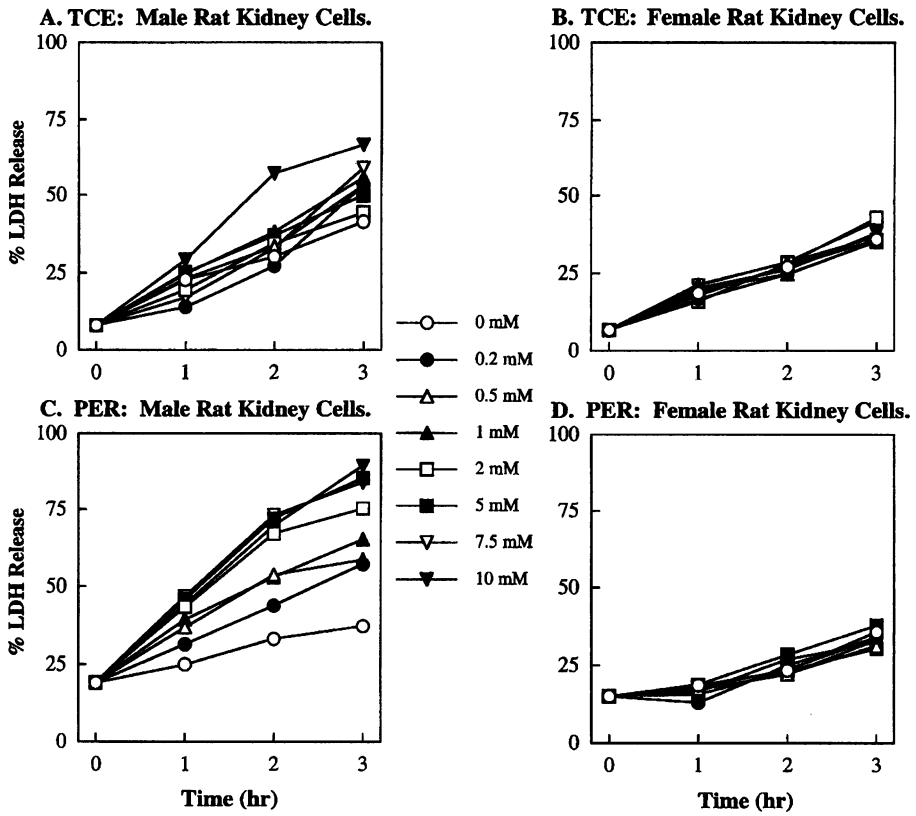

Figure 1. Time and concentration dependence of cytotoxicity of TCE and PER in isolated kidney cells from male and female rats. Isolated renal cortical cells (2 to 3 × 10^6 cells/mL) were obtained by collagenase perfusion of kidneys from male or female F344 rats. Cell suspensions were incubated with the indicated concentrations of TCE (A, B) or PER (C, D) for up to 3 hr at 37°C on a metabolic shaking water bath. TCE and PER were dissolved in ethanol (final ethanol concentration < 1%). At the indicated times, aliquots were removed for measurement of lactate dehydrogenase (LDH) release. Results are the means of incubations from 3 or 4 separate cell preparations.

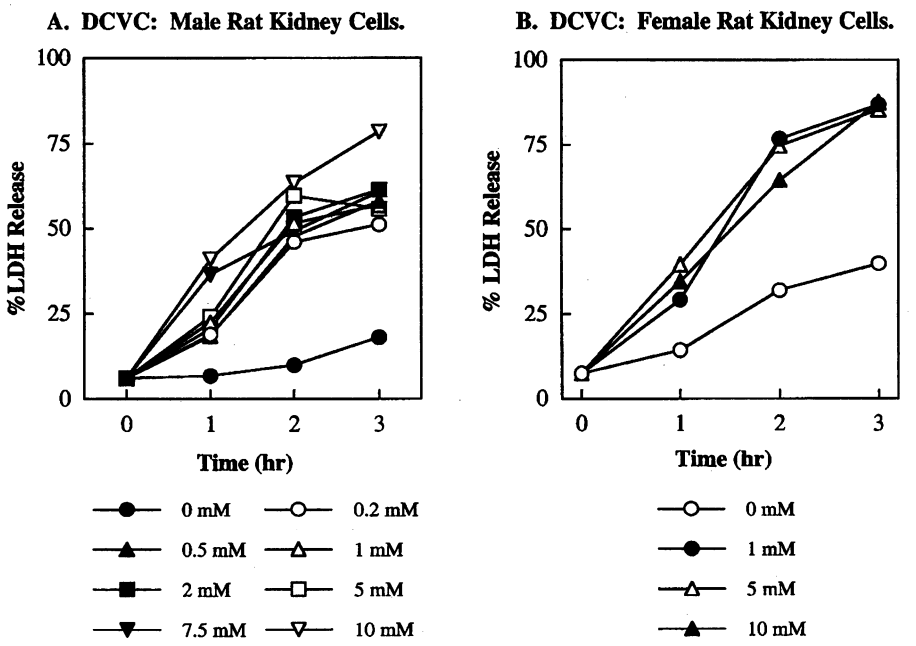

Figure 2. Time and concentration dependence of cytotoxicity of DCVC in isolated kidney cells from male and female rats. Isolated renal cortical cells (2 to 3 × 10^6 cells/mL) were obtained by collagenase perfusion of kidneys from male or female F344 rats. Cell suspensions were incubated with the indicated concentrations of DCVC for up to 3 hr at 37°C on a metabolic shaking water bath. At the indicated times, aliquots were removed for measurement of lactate dehydrogenase (LDH) release. Results are the means of incubations from 3 or 4 separate cell preparations.

was not diminished by preincubation of cells with AOAA, suggesting that other bioactivation mechanisms for DCVC may also be present in the human kidney.

Anders and colleagues (*146*) recently demonstrated the catalytic function of the β-lyase in human volunteers anesthetized with sevoflurane, which is metabolized to compound A (2-[fluoromethoxy]-1,1,3,3-pentafluoro-1-propene). Compound A is nephrotoxic in rats (*147–154*) and undergoes β-lyase-dependent metabolism with recovery of GSH conjugates and mercapturates in bile and urine, respectively (*149,155*). Iyer et al. (*146*) identified two metabolites of compound A, 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid and 3,3,3-trifluorolactic acid, in the urine of human volunteers anesthetized with sevoflurane that could only arise by action of the β-lyase, thus demonstrating the *in vivo* function of the β-lyase in humans.

The discussion in the article on "Metabolism of Trichloroethylene" (*1*) of DCVC as being a branch point in the metabolism of TCE by the GSH pathway is relevant to an understanding of TCE-induced renal toxicity. DCVC can either undergo *N*-acetylation to produce the mercapturate, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (NAcDCVC), which is excreted in the urine, or it can be bioactivated by the β-lyase or other enzymes to form reactive species that produce toxicity. Additionally, NAcDCVC can be deacetylated within renal proximal tubular cells to regenerate DCVC. The toxicological significance of this was demonstrated in studies that showed that both NAcDCVC and DCVC are toxic *in vivo* and to *in vitro* renal preparations (*156,157*). A scheme summarizing these alternative fates of DCVC is shown in Figure 3.

The ability of other enzymes besides the β-lyase, such as the L-α-hydroxy (L-α-amino) acid oxidase (HAO) and the flavin-containing monooxygenase (FMO) cysteine conjugate *S*-oxidase (*S*-oxidase), to bioactivate DCVC is discussed in detail in the article on "Metabolism of Trichloroethylene" (*1*). The β-lyase is the primary enzyme responsible for bioactivation of DCVC. The toxicological relevance of this has been demonstrated both in whole-animal *in vivo* studies (*131*) and in various *in vitro* renal preparations (*138,142, 158,159*) by showing that alterations in β-lyase activity, such as inhibition with AOAA or stimulation with 2-keto acids, correspondingly alters DCVC toxicity.

In spite of the undisputed importance of the β-lyase in cysteine conjugate-induced nephrotoxicity, several studies have provided evidence that additional enzymatic activities can bioactivate DCVC and other haloalkenes and haloalkanes and lead to toxicity. In two studies on the immunohistochemical localization of the β-lyase in the renal tubular epithelium, some discrepancies were noted between enzyme localization and the specific renal cell population that was most susceptible to cysteine conjugate-induced injury. Although MacFarlane et al. (*160*) showed a correspondence between the nephron segments primarily involved in the β elimination of DCVC and TFEC and those most susceptible to HCBD-induced cellular necrosis, Jones et al. (*161*) found that distribution of the β-lyase activity within the proximal tubules did not correspond with the nephron segment that was the most sensitive to DCVC and PCBC.

Although some role for the HAO in DCVC bioactivation is supported by *in vitro* experimental data (*162,163*), this pathway functions only in the rat, since the enzyme activity is absent from the kidneys of most mammalian species, including humans. Stronger evidence exists for a role for the *S*-oxidase. Several studies have demonstrated that DCVC sulfoxide (*159,164*) and other sulfoxides of cysteine conjugates or mercapturates (*165–167*) are more potent nephrotoxicants than the corresponding cysteine conjugates. The presence of the *S*-oxidase activity in human kidney has not been studied yet, but identification of the activity as possibly catalyzed by FMO1A1 (*168*) suggests that it will likely be present in human kidney as well. Nonetheless, the available data indicate that most of the renal bioactivation of DCVC and similar cysteine conjugates is mediated by the β-lyase.

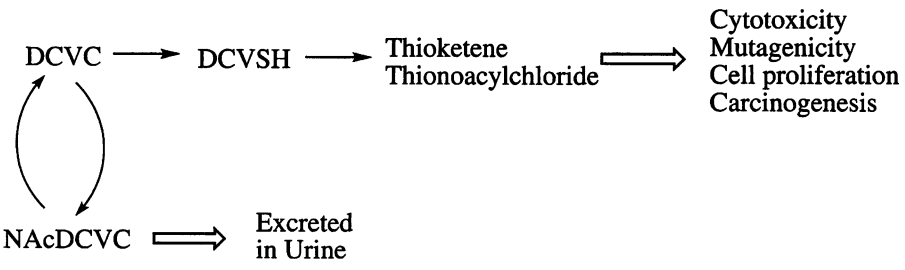

Figure 3. Generalized scheme showing fates of DCVC in the kidneys. DCVC [*S*-(1,2-dichlorovinyl)-L-cysteine] may be metabolized by the cysteine conjugate β-lyase to *S*-(1,2-dichlorovinyl)thiol (DCVSH) or the mercapturate *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (NAcDCVC). Balance between fluxes of the β-lyase, *N*-acetylation, and deacetylation will determine the toxic response in the kidneys.

Acute mechanisms of proximal tubular cytotoxicity. Limited experimental data with TCE on its biochemical modes of action in the kidneys are available. Hence, most of the available information on the biochemical effects of TCE in renal proximal tubular cells must be inferred from data on DCVG and DCVC. These two conjugates have been used extensively as models to study biochemical mechanisms of renal cellular injury. The realization that these chemicals are actually formed *in vivo* from TCE and GSH in both animals and humans and are not just model compounds adds significance to the studies on DCVG and DCVC for risk assessment of TCE.

Oxidative stress. An imbalance between intracellular reductants and oxidants in favor of oxidants is termed oxidative stress. This has been implicated as a mechanism of toxicity for a vast number of chemicals, in several pathological states, and in aging (*169*).

Several studies have associated either TCE (*128*) or DCVC (*142,170–175*) with causing oxidative stress after exposure of renal cells to these chemicals. Primary biochemical components of the oxidative stress response include GSH oxidation or depletion, lipid peroxidation, and oxidation or alkylation of protein sulfhydryl groups. Each of these processes, when altered, leads to secondary effects that may then cause cytotoxicity. Often, however, cytotoxicity can be dissociated from processes such as lipid peroxidation. This can be demonstrated by showing that an inhibitor of lipid peroxidation does not protect cells from the cytotoxicity or cellular necrosis induced by DCVC. Hence, such responses can be considered epiphenomena that are not causally related to cellular injury. It is likely that oxidative stress plays some role in DCVC-induced nephrotoxicity, but lipid peroxidation is probably a consequence rather than a cause of cellular injury.

Disturbances in calcium ion homeostasis. Perturbations in intracellular Ca^{2+} ion homeostasis have been implicated in the toxicity of a large number and variety of xenobiotics. Cells such as those in the renal proximal tubular epithelium maintain free Ca^{2+} concentrations in the cytosol in the range of 0.1 μM, whereas extracellular Ca^{2+} concentrations are in the range of 1 mM. Hence, there is a 10,000-fold concentration gradient of Ca^{2+} ions across the plasma membrane. Consequences of raises in intracellular Ca^{2+} concentrations induced by DCVC include inhibition of mitochondrial metabolism and function (*138,174,176*), severe mitochondrial damage (*177*), poly(ADP)-ribosylation of nuclear proteins and DNA double-strand breaks (*124*), and changes in cytoskeletal protein structure and plasma membrane blebbing (*171,177*). Renal epithelial cells contain multiple pools of Ca^{2+} ions,

each differentially regulated. Data suggest that these pools are not all equally sensitive to DCVC. Rather, it appears that the mitochondrial Ca^{2+} ion pool is the most sensitive to disturbances (*124,138,174,177*).

The changes in cellular function that occur as a consequence of the changes in Ca^{2+} ion distribution may be attributed, at least in part, to the activation of Ca^{2+}-dependent degradative enzymes, such as proteases, endonucleases, and phospholipases. The critical importance of Ca^{2+} ion homeostasis, and the data that clearly show early effects of DCVC metabolites, suggest an important role for disturbances in Ca^{2+} ion homeostasis in the biochemical mechanism of action of DCVC.

Mitochondrial dysfunction. The mitochondria were implicated a number of years ago as a primary target site within the cell for nephrotoxic cysteine *S*-conjugates such as DCVC. Parker and colleagues (*178–180*) utilized rat liver mitochondria for their pioneering investigations on the mitochondrial toxicity of DCVC with the thinking that even though the liver is not affected by administration of DCVC, isolated liver mitochondria can be obtained more easily and in greater quantities and are typically more functionally intact than isolated kidney mitochondria. Although these points are true, it should not be taken *a priori* that responses seen in liver and kidney mitochondria will be the same. Furthermore, by use of liver mitochondria as a model system, the impression is created that this is a normal *in vivo* target site, which is incorrect. As discussed in the section on interorgan metabolism in the article on "Metabolism of Trichloroethylene" (*1*), the liver cannot take up GSH conjugates and has efficient means for secretion of GSH, cysteine, and *N*-acetylcysteine conjugates into bile or plasma. Hence, liver mitochondria will not be exposed to DCVC nor will they exhibit diminished function as a consequence of exposure to DCVC, even though the liver does have β-lyase activity.

More recently, several studies on the mechanism of action of DCVC and related cysteine conjugates have focused on the mitochondria and have shown that mitochondrial dysfunction is an early event in the course of exposure to these compounds that may be causally associated in many cases with cellular injury (*138,173,174,181*). Only one study looked at the effects of TCE on renal cortical mitochondria (*81*), where TCE was shown to modestly inhibit state 3 respiration and to increase state 4 respiration, indicative of membrane damage and uncoupling. Unlike TCE, DCVC does not uncouple mitochondria but inhibits state 3 respiration by specifically inhibiting several sulfhydryl-containing enzymes (*81,174,182,183*). Experiments on the coupling site specificity of the inhibition of mitochondrial respiration showed that site II, the succinate dehydrogenase complex, is the most susceptible to DCVC (*138,173, 174*) and that this is related to inhibition of the enzyme (*174,175*).

Formation of covalent adducts with proteins and other macromolecules in mitochondria have been documented (*182,184, 185*). Some of the covalent binding to mitochondrial proteins occurs through formation of mixed disulfides (*182*), although other nucleophilic sites are also targeted. Covalent binding of DCVC is largely dependent on its metabolism by the β-lyase, as AOAA blocks much of the binding. The fraction of mixed disulfides as opposed to adducts through other nucleophilic groups, such as the ε-amino group of lysyl residues, differs with different cysteine conjugates, indicating that the chemical nature of the reactive metabolite from various cysteine conjugates also differs. The correlation between covalent binding and mitochondrial dysfunction also does not clearly correspond with the extent of metabolism (*182*). Hence, it is likely that formation of covalent adducts with mitochondrial proteins is only one mode of action.

The mitochondrial genome is another potential target of nephrotoxic cysteine conjugates (*186*), where cysteine conjugates inhibit macromolecular synthesis and produce DNA damage.

Protein alkylation. In addition to mitochondrial proteins that are alkylated by reactive metabolites of DCVC and other cysteine conjugates, cytosolic proteins have been identified as specific targets. Eyre et al. (*187*) found that both TCE and DCVC, administered *in vivo*, produced acid-labile adducts with protein. Pretreatment with either diethylmaleate, which depletes cellular GSH content, or the β-lyase inhibitor AOAA inhibited adduct formation with TCE, indicating that most of the renal adduct formation from TCE was due to metabolism by the GSH conjugation pathway and not by P450. In a companion study (*188*), they also showed that whereas the extent of metabolism and covalent binding in mice was greater than in rats, it is the rats that are more sensitive to TCE-induced nephrotoxicity. However, rates of cell replication were correspondingly higher in mice. Hence, they concluded that other factors besides covalent adduct formation must contribute to the induction of renal carcinogenesis by TCE.

It is often difficult to associate the finding of covalent adducts with proteins and functional changes in the cell. However, Lock and Schnellmann (*189*) identified cytosolic glutathione reductase and mitochondrial lipoyl dehydrogenase from rat renal cortex as specific targets of reactive species generated from β-lyase-dependent metabolism of DCVC. Chen et al. (*190*) and Bruschi et al. (*191*) showed that one consequence of alkylation of cellular macromolecules by metabolites of cysteine conjugates is the transcriptional activation of stress proteins, such as the 70-kDa and 60-kDa heat shock proteins (hsp70 and hsp60). Hence, covalent adduct formation can lead to regulatory changes in the renal cell. In another study from Stevens and colleagues (*144*), DCVG and DCVC were shown to form covalent adducts with proteins from human proximal tubular cells. Thus, the same biochemical mechanisms observed in rodent kidney cells also occur in the human kidney.

Renal repair processes. A potential response to perturbations caused by exposure to cytotoxic agents is the induction of repair processes. One repair response to agents that form adducts with or damage DNA is the induction of UDS (*122,123*). Wallin et al. (*192*) observed changes in expression of certain cellular proteins after DCVC-induced cellular injury and the induction of nephrogenic repair. Cytokeratins are characteristic marker proteins that are expressed in normal, differentiated renal proximal tubular cells and other differentiated epithelial cells. After cellular regeneration begins, expression of cytokeratins decreases and expression of vimentin, which is normally characteristic of endothelial cells, increases. Corresponding with this change in expression is an increase in DNA synthesis as cellular proliferation occurs. In a study by Ward et al. (*193*) of vimentin expression in the kidneys of both control and nephrotoxicant-treated male rats of various ages and in human renal-cell carcinomas, increased vimentin expression was noted in regenerating renal tubular lesions of toxicant-treated rats and in most human renal-cell carcinomas and latent preneoplastic or neoplastic renal tubular lesions that were found incidentally at autopsy. Hence, the repair–proliferation response also occurs in human kidney and is associated with both regeneration after toxicant damage and in development of neoplasias.

Schnellmann and colleagues (*194–196*) have developed and validated an *in vitro* model of renal proximal tubule regeneration using primary cultures of proximal tubular cells from rabbit kidney. From these and the studies described above, it is clear that DCVC can induce damage that leads to a repair response. This repair response is characteristic of renal tissue in both rodents and humans and is therefore relevant to a consideration of human exposure to TCE.

Alterations in gene expression and cell proliferation. Another aspect of the repair and proliferative responses to nephrotoxicants or neoplasia is changes in gene expression that can occur and that may underlie these responses. In addition to demonstrations that

DCVC induces renal-cell repair and proliferation (*188,192*), activation of specific genes that have been associated with regulation of cellular growth and differentiation, such as hsp60 and hsp70 (*190,191*), c-*fos* and c-*myc* (*197,198*), and *gadd* 153 (growth arrest and DNA damage) (*199*), have been found. Hence, these data show that reactive metabolites generated from DCVC metabolism can alter the expression of critical genes that are involved in the control of cell growth and differentiation. A detailed dose- and time-dependence study of these responses in relation to other effects of DCVC has not, however, been performed. It is thus difficult to assess the relevance of these effects with respect to exposure to TCE.

Contribution of Different Modes of Action to TCE-Induced Kidney Tumorigenesis

It is likely that multiple modes of action may be important in TCE-induced kidney cancer and that different modes or combinations of modes of action may be important at high or low doses of TCE. For example, several mechanisms of acute proximal tubular necrosis may occur, and both genotoxic and nongenotoxic mechanisms may be involved in the development of kidney tumors. A schematic summary of renal effects of TCE that are mediated through the GSH conjugation pathway are shown in Figure 4. One must consider that the various cytotoxic, repair, and proliferative responses represent a continuum, and that the relative importance of different responses will depend on the dose of the reactive species and on several factors relating to renal cellular function. For example, DNA repair processes, oncogene activation, and cellular transformation require intact cellular structure and an adequate supply of ATP for protein synthesis. Hence, if cells are exposed to very high doses of DCVC that produce extreme mitochondrial dysfunction, it is likely then that the tissue will not be competent to undergo repair and proliferation.

From the available data, one can conclude that exposure of renal cells to high doses of DCVC will produce oxidative stress, protein and DNA alkylation, and mitochondrial dysfunction. As a consequence of inhibition of active transport processes and marked ATP depletion, cytotoxicity will occur and result in acute tubular necrosis. At lower doses, in contrast, it is likely that mild changes in mitochondrial function and oxidative stress as well as selective alkylation of protein and DNA will occur, and that these effects will lead to changes in homeostatic processes in the cell that will ultimately alter gene expression and cell growth. A task for investigators will be to delineate the conditions under which the various responses can occur.

Questions and Research Needs

Renal Concentration of Toxic Metabolites

Better methods are needed to quantitate reactive species that are generated during TCE metabolism, particularly in the β-lyase pathway. This will also improve utility of *in vitro* studies by allowing more accurate comparisons of *in vivo* and *in vitro* studies and will help in the validation of PBPK models. Urinary excretion of mercapturic acids has been used as a marker for the function of the β-lyase pathway, indeed as a marker of exposure (*200*). However, many investigators have misused this information to make conclusions regarding the flux through the β-lyase pathway relative to that through the P-450 pathway, stating that the relative flux through the β-lyase pathway is more than three orders of magnitude lower than that through the oxidative pathway. This conclusion is based on comparisons of urinary NAcDCVC, TCA, and trichloroethanol (TCOH).

It must be remembered that NAcDCVC represents only a fraction of the TCE that is metabolized through the GSH conjugation pathway. After being metabolized to DCVC, two fates are possible: *N*-acetylation or metabolism by the β-lyase or another enzyme that generates a reactive metabolite. Eyre et al. (*187*) and van Welie et al. (*200*) have suggested that protein and DNA adducts should be used as markers of flux of reactive metabolites from TCE through DCVC. Problems with this approach are that many of these adducts are chemically unstable, so that recovery during analytical procedures

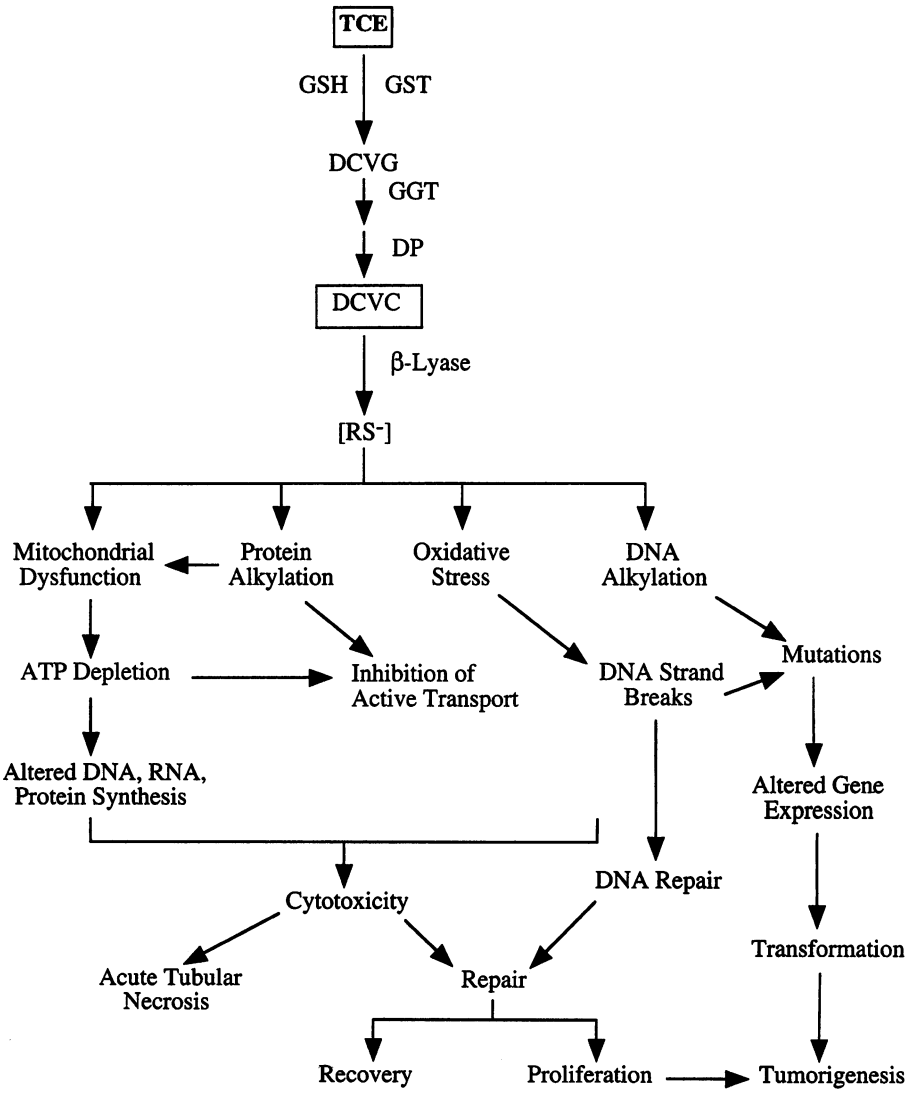

Figure 4. Summary scheme of the postulated modes of action of TCE via the GSH conjugation pathway for nephrotoxicity and nephrocarcinogenicity. The scheme summarized demonstrated and hypothesized modes of action of TCE in mammalian kidney, showing the various intracellular targets and the interplay between them in ultimately causing nephrotoxicity or nephrocarcinogenicity. Abbreviations used: DP, dipeptidase; RS^-, reactive thiol and subsequent species generated from β-lyase-catalyzed metabolism of DCVC.

may be incomplete and variable. The primary purpose for obtaining better data on the flux through the β-lyase pathway is to assess if enough of the reactive metabolite generated from DCVC is produced at typical exposure doses of TCE. Recently, Bruckner and colleagues (*83*) have made progress in this area by estimating tissue concentrations of TCE with a PBPK model. Additional efforts in this area will help clarify the quantitative significance of the β-lyase pathway and the renal modes of action in TCE-induced carcinogenesis.

Male and Female Renal Cancer Differences in Rats versus Similarities in Biochemistry

The most recent biochemical data on TCE and similar aliphatic hydrocarbons such as PER that are being obtained by various investigators need to be correlated with cancer data to determine if correlations between biochemistry and toxic responses can be made. Although this has been done to some extent, additional studies are needed. As discussed above, the different modes of action in the kidney, which range from cytotoxicity to DNA damage to alterations in gene expression and stimulation of proliferation, should be viewed as a continuum. Dose dependencies for these various responses need to be determined and correlated with tumor incidence and susceptibility in male and female rodents and humans.

Human GSH *S*-Transferase Activity for Conjugation with TCE

These data will allow more complete assessment of the quantitative importance of this pathway in humans and will allow better comparisons between data from laboratory animals and those from humans. Data are currently available for this pathway in cultured human hepatocytes and human liver and kidney cytosol and microsomes [see article on "Metabolism of Trichloroethylene" (*1*) and Lash et al. (*201*)]. Complete assessment of these results will allow a true, quantitative comparison of metabolic rates in kidney and liver from humans and rodents.

Pharmacokinetic and biochemical study of blood and urine from human volunteers exposed to TCE by inhalation showed that DCVG can be detected in the blood, demonstrating function of at least the first step of the GSH conjugation pathway in humans (*202*). Function of the β-lyase in humans for TCE has not been directly demonstrated. However, Völkel et al. (*203*) demonstrated recovery of DCA from PER in blood of rats, but not humans, exposed to PER by inhalation. In this case, DCA can arise only by β-lyase-dependent metabolism. The authors concluded that this provided evidence of significantly higher flux of PER through the β-lyase pathway in rats than in humans. Kharasch et al. (*204*) demonstrated function of the β-lyase pathways in rats exposed to compound A by quantifying recovery of 3,3,3-trifluoro-2-(fluoromethoxy)propanoic acid in urine. A similar, although not as extensive, analysis in humans exposed to compound A (*205*) indicated flux through the bioactivation pathway was 6-fold greater in rats than in humans. Again, these data suggest that use of rodent data for human health risk assessment likely overestimates the risk to humans.

GST Isozyme Specificity for GSH Conjugation of TCE

The significance of species-, sex-, and tissue-dependent differences in expression of GST isozymes and genetic polymorphisms in determining overall metabolism and toxicity for many chemicals is becoming increasingly apparent. Hence, there is a need, in both rodents (rats, mice) and humans, to quantify the activity of different GST isozymes toward TCE. A recent study by Cummings et al. (*206*) shows that rat kidney proximal tubular cells express GSTα but not GSTμ or GSTπ, and kinetic and inhibitor studies show that GSTα1-1 is the primary isoenzyme in rat kidney that catalyzes GSH conjugation of TCE. Similar studies have not yet been conducted in human kidney cells. However, Cummings et al. (*207*) found that unlike rat proximal tubular cells, freshly isolated and primary cultures of human proximal tubular cells express GSTA, GSTP, and GSTT, suggesting that the ability of human kidney to catalyze GSH conjugation of TCE may differ significantly from that of rat kidney.

Relative *in Vivo* Rates of β-lyase versus *N*-Acetyltransferase/Deacetylase in DCVC Metabolism

These data are important for allowing us to better track the fate of DCVC in renal tissue and, hence, to determine the overall flux of TCE through this pathway. The primary method for quantifying flux through the β-lyase pathway has been measurement of mercapturates. However, as discussed above, several competing reactions occur whose rates we currently do not or cannot accurately determine. The presence of deacetylation reactions further complicates the situation and makes the interpretation of mercapturate formation less clear. The key rates to be determined, therefore, are those for the β-lyase, the *N*-acetyltransferase, and the deacetylation reaction. Green et al. (*208*) have reported that in human kidney, metabolism by the *N*-acetyltransferase is two orders of magnitude greater than that by β-lyase. However, the rates of DCVG formation reported by Green et al. (*208*) in rodents and in human tissue are more than two orders of magnitude lower than those reported by Lash et al. (*81,82*). Hence, continued research in this area is clearly warranted.

Studies with the chemically related compound PER (*203*), as discussed above, suggest that the biotransformation rate of PER by the β-lyase pathway is significantly higher in rats than in humans. The authors concluded that use of rat tumorigenicity data for human health risk assessment of PER may overestimate human tumor risks. A second study (*209*) examined the formation of protein adducts in kidney, liver, and blood of rats, and in human blood after PER inhalation and found much lower levels of adducts in human blood than in rat blood, again suggesting that toxicity is greater in rats than in humans.

Role of Renal Cytochrome P450 in TCE-Induced Renal Toxicity and Carcinogenesis

Studies are needed to quantitate rates of renal P450 metabolism of TCE in rats, mice, and humans. Although most of the available data with TCE in the kidney indicate that the renal effects of TCE arise from generation of DCVC and subsequent reactive species, the oxidative pathway has not been thoroughly investigated in the kidneys.

Further Research into the Modes of TCE- and DCVC-Induced Renal Toxicity

Although much data have been accumulated on various modes of action in the kidneys, a precise sequence of events cannot clearly be constructed. A fine line may exist between cytotoxic events and those that lead to renal cellular repair and/or proliferation. The latter may allow transformation and carcinogenesis to occur. Defining conditions that produce cytotoxicity, sublethal alterations and repair, proliferation, and transformation is critical to understanding mode of action. Furthermore, this is necessary to provide an appropriate and correct human health risk assessment. Studies defining dose and time dependencies for the different responses are warranted.

The recent work on the VHL gene and its role in renal cancer suggests that mutations in the VHL gene may be an important mode of action for many examples of chemically induced renal cell cancer. A more detailed analysis and comparison of VHL mutational spectra, therefore, in TCE exposed and non–TCE-exposed cases of renal cell carcinoma is warranted to allow a full and accurate appraisal of the biological significance of this mode of action.

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